Indian J. Phys. **73B** (4), 671-677 (1999)

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IJP B

— an international journal

Effect of hydrogen bonding on the electronic absorption spectra of some nucleic acid bases

E Waghorne, E Duggan, G Will, D Fitzmaurice and S Mukherjee[†‡]

Department of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland

[†] Department of Physical Chemistry, Indian Association for the Cultivation of Science, Calcutta-700 032, India

Received 15 March 1999, accepted 11 May 1999

Abstract The effect of hydrogen bonding on the absorption spectra of some proton donor nucleic acid bases such as uracil, thymine, 5-bromouracil (BrU) and 5-aminouracil (AmU) has been studied with acceptor bases like NaOH and piperidine in different protic solvents. It is observed that the $\pi\pi^*$ absorption bands of the proton donor shift toward longer wavelengths due to hydrogen bond formation and, using this change of absorption spectra, that the equilibrium constant between the proton donor and acceptor can be evaluated From our findings, it is concluded that proton donating power decreases in the order BrU > uracil > thymine > AmU. Some conclusions are drawn concerning the mechanism of hydrogen bonding

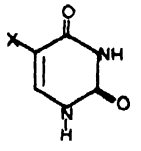
Keywords : Spectroscopic studies, hydrogen bonding, nucleic acid bases

PACS Nos. 82 30 Nr, 82 80 Ch, 31.70.Dk

The electronic spectra of a molecule may be altered by the formation of hydrogen bonds if the chromophoric part of the molecule is perturbed by the hydrogen bond formation, and this is often accompanied by spectral changes due to $\pi\pi^*$ and $n\pi^*$ transitions. Several authors have discussed hydrogen bonding in aromatic amines acting either as proton donor or as proton acceptor [1-3]. When aromatic amino or imino groups enter into hydrogen bond formation with a suitable acceptor, the $\pi\pi^*$ absorption band of aromatic compounds show a red shift. It was recognized by various workers that the electronic absorption spectra of organic molecules are not independent of the solvent employed. Considering the solvent effect, the absorption maxima is also shifted towards longer wavelength with increasing dielectric constant of the solvent. It is fairly well established that "inductive" and "dispersive" effects are primarily responsible for this red shift. The red shift of $\pi\pi^*$ transition in a base can be attributed to the electron distribution with enhanced electron density in the more peripheral portion of the molecule where the presence of electrons are more effective for hydrogen bond formation.

‡ Author for correspondence.

In all forms of life, there are only two forms of nucleic acid, ribonucleic acid and deoxyribonucleic acid. Uracil and thymine are two important heterocyclic bases present in both the nucleic acids (Figure 1). Nucleic acids are of fundamental importance in controlling the reproduction and growth in living system. Moreover, nucleic acids are the basis of storage, transmission and expression of genetic information. Hence, any interaction or damage caused to the nucleic acid will have important consequences.



X = H, CH_3, Br or NH_2

uracil

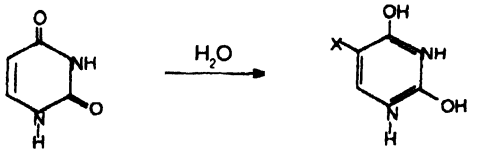
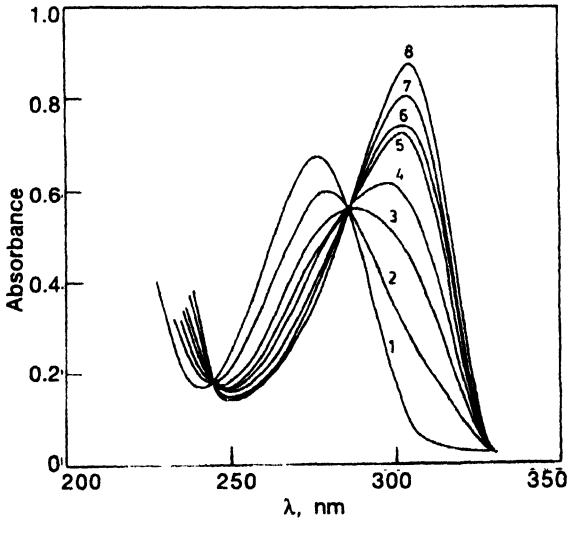


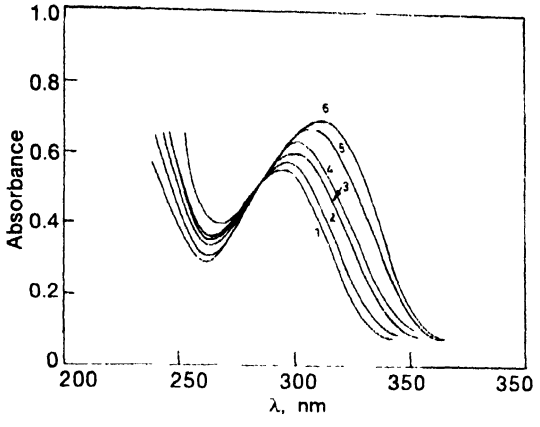
Figure 1. Structural formula of nucleic acid bases

The usefulness of ultraviolet spectroscopy in studying hydrogen bonding interaction has been recognized [4]. In this paper, the electronic absorption spectra of uracil, thymine, 5-bromouracil (BrU) and 5-aminouracil (AmU) in different protic solvents are described. The object of the present investigation was to study if the imino hydrogen (>N-H) present in all these nucleic acid bases (NuB) can enter into hydrogen bond formation with bases like NaOH and piperidine.



2 (a).

The samples of nucleic acid bases (NuB) uracil (98%), thymine (97%), 5-bromouracil (BrU, 99%) and 5-aminouracil (AmU, 98%) all were obtained from either Sigma or Aldrich and were used as received. Ethanol and ethylene glycol (EG, Merck, spectroscopic grade) were dried and distilled before use. Analytical grade NaOH and piperidine were used as bases. Triply distilled water was used throughout.



2 (b)

Figure 2. Absorption spectra of (a) thymine and (b) 5-aminouracil in ethanol at different concentrations of NaOH . Range of [NaOH] . (a) (1-8) . 1 5 – 8 5 × 10^{-4} mol dm^{3} and (b) (1–6) 0 5 – 4 0 ×10^{-4} mol dm^{-3}

The electronic absorption spectra were carried out using a Hewlett Packard absorption spectrophotometer, model 8452A, attached to a power PC Macintosh 4400/200 computer. The concentration of NuB were maintained between 6-8 × 10^{-5} mol dm^{-3} and kept fixed in each set of experiments. The concentrations of proton acceptor bases were increased in steps accordingly.

When an increasing amount of a proton acceptor base like NaOH or piperidine was added to a solution containing nucleic acid bases (NuB), it was observed that the absorption bands characteristic of free NuB became progressively less prominent and at the same time, a new band appearing on the longer wavelength side became stronger. It is well known that when aromatic amino compounds enter into hydrogen bond formation, the electronic absorption band of the aromatic compound shows a red shift [5, 6]. Hence, when the concentration of the proton acceptor base was several times larger than that of the proton donor, the proton donor might be assumed to exist completely in the hydrogen bonded state. The peak was associated with the characteristic $\pi\pi^*$ band of the proton donor, NuB. The ground state interaction of NuB bases was investigated using the assumption that they form 1 :1 hydrogen bonded complexes with NaOH or piperidine. Moreover, NuB are assumed to be proton donors whereas NaOH and Piperidine are acceptor bases (PAB). In Figures 2a and 2b two typical examples of the absorption spectra of BrU and AmU in ethanol both in presence and absence of NaOH are given. The position of these absorption bands of different NuB are shown in Table 1. As can be seen in Figure 2, the absorption of BrU in the short wavelength region decreased upon addition of NaOH owing to the decrease in its equilibrium concentration, while new absorption band appears in the long wavelength region owing to the formation of hydrogen bonded complex. In the case of AmU, the intensity of the band increases with a red shift due to the formation of the complex as shown in Figure 2b. An isosbestic point is also observed in every

case as shown in Figures 2(a, b) indicating the presence of an acid-base equilibrium between the reacting species.

Table 1. Frequency shift ($\delta\nu = \nu_b - \nu_f$) on hydrogen bond formation (frequencies in cm^{-1}).

System	ν_f	ν_b	$\delta\nu$
BrU	36364	32787	3577
uracil	36765	33557	3208
thymine	37736	34483	3253
AmU	33557	32258	1299

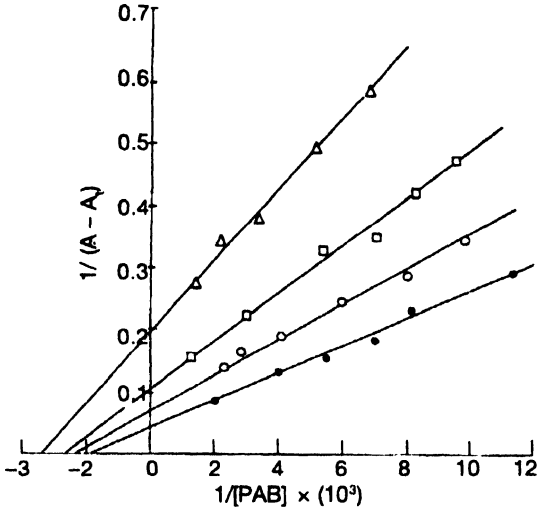


Figure 3(a).

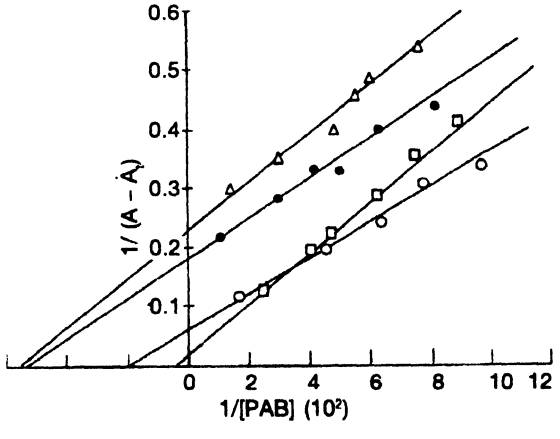


Figure 3(b)

Figure 3. Plots of $\frac{1}{A - A_f}$ *vs* $\frac{1}{[PAB]}$ for the determination of equilibrium constant ; (a) : Δ(BrU + water), □ (BrU + EtOH), o(uracil + EtOH) and •(thymine + water) (b) Δ(BrU + piperidine + EG), •(thymine + piperidine + EG), □(AmU + NaOH + EtOH) and o (AmU + water + NaOH).

The method of Ketelaar *et al* has been followed in the present investigation in calculating the association equilibrium constant of the hydrogen bonded complexes (K_g) from eq. (1) [7, 8].

$$\frac{1}{A-A_f} = \frac{1}{[PAB]} \cdot \frac{1}{A_b - A_f} \cdot \frac{1}{K_g} + \frac{1}{A_b - A_f}, \quad (1)$$

where A_f and A_b stand for the absorbance of free proton donor (NuB) and the hydrogen bonded complex, respectively and A that of a solution of donor where the concentration of the proton acceptor base PAB is [PAB]. On extrapolating the straight line obtained from $\frac{1}{A-A_f}$ *vs* $\frac{1}{[PAB]}$ plot to the point where $\frac{1}{A-A_f} = 0$, K_g was obtained for all the cases as $K_g = -\frac{1}{[PAB]}$. The plots are shown in Figure 3 and the K_g values are displayed in Table 2. The plots are linear in all cases indicating 1 : 1 complex formation in these systems.

It can be seen from Table 1 that the frequency shift caused by the hydrogen bonding decreases almost in the order BrU > uracil > thymine > AmU in all the protic solvents used here. This order of frequency shift is in complete accordance with that of the equilibrium constant given in Table 2 only in water in presence of NaOH. It seems certain from the results that interaction of BrU with NaOH in water is the strongest and AmU with piperidine in alcohols is the weakest. It is also noted that AmU does not have any interaction in ethylene glycol (EG). This is consistent with the fact that AmU is a stronger base and weaker proton donor, due to the presence of NH_2 compared to those of thymine and BrU. The CH_3 group present in thymine is known to be practically inactive, the C-H compounds are not usually hydrogen bonding acids [10] and Br group is electron withdrawing. On the otherhand, the proton acceptor which forms the strongest hydrogen bond exerts the larger perturbation on the absorption spectrum of the proton donor. Our results (Table 2) show that the interaction is stronger when NaOH is used as acceptor base. It is also noted that with the same proton acceptor base, the frequency shift of the $\pi\pi^*$ bands of the proton donors due to hydrogen bonding is quite systematic. That is hydrogen bonding shift decreases with basicity of the proton donor. On the basis of equilibrium constant measurements, it can be said that the proton donating power has the order BrU > uracil > thymine > AmU.

Table 2. Equilibrium constant (K_g) of hydrogen bond formation in different solvents

Proton donor	Acceptor	Solvent	K_g, dm^3/mol	Range of [acceptor] mol dm^{-3}
BrU	NaOH	Water	2.7×10^3	$1.1 - 4.8 \times 10^{-4}$
BrU	NaOH	EtOH	2.4×10^3	$2.5 - 8.6 \times 10^{-4}$
BrU	piperidine	EG	5.8×10^2	$1.0 - 6.9 \times 10^{-4}$
Uracil	NaOH	Water	2.4×10^3	$1.6 - 9.5 \times 10^{-4}$
Uracil	NaOH	EtOH	2.1×10^3	$1.5 - 9.8 \times 10^{-4}$
Uracil	piperidine	EG	4.2×10^2	$1.5 - 8.0 \times 10^{-3}$
thymine	NaOH	Water	2.0×10^3	$0.5 - 9.2 \times 10^{-4}$
thymine	NaOH	EtOH	2.6×10^3	$1.0 - 8.5 \times 10^{-4}$
thymine	piperidine	EG	5.6×10^2	$1.0 - 9.0 \times 10^{-3}$
AmU	NaOH	Water	2.0×10^2	$0.5 - 8.5 \times 10^{-3}$
AmU	NaOH	EtOH	0.5×10^2	$1.5 - 9.5 \times 10^{-3}$

Due to the presence of NH_2 group in AmU, the electron density around nitrogen atom will increase thereby increasing the basicity of AmU. For the formation of a stable hydrogen bond N – H – B, the only requirement of the acceptor is that the charge distribution of the N–H bond orbital be such as to have the proton sufficiently unscreened. Hence, the interaction is weaker and the equilibrium constant with AmU is lower as expected due to the increased electron density around nitrogen atom and lowering the equilibrium constant by about one order of magnitude. This is due to the lowering of the electrostatic energy. The delocalisation or charge transfer force means the stabilization due to electron migration from a non-bonding orbital of the proton acceptor base to N-H anti-bonding orbital of the proton donor. In the present systems, both the charge transfer force and the electrostatic force have contributions for the stabilisation of hydrogen bonding.

It may be argued also that under the influence of the electron donor bases, the σ-electrons associated with N–H bond will be pushed towards the nitrogen atom and the resulting increase in the electron density around the nitrogen atom will lead to a decrease in the binding energy of the sp^2 hybridized lone pair of nitrogen. As a result, their interaction with the ring π-system will increase accounting for the shift and enhanced intensity of the spectra of the hydrogen bonded species [4]. It is to mention here that because of the presence of electron deficient nitrogen atom and owing to the high polarity of the imino (>NH) group, NuB can preferentially act as proton donor.

One more point to mention is that we are unable to detect any spectral change by the addition of bases like triethylamine (TEA) or piperidine in acetonitrile and acetone containing NuB. Moreover, it is observed that the interaction is always stronger when water is used as solvent and weaker when ethylene glycol is used. This seems to indicate that there may be some interaction between protic solvents and NuB as shown in Figure 1. The interaction is expected to be stronger now since hydroxyl group is much more stronger proton donor than imino group.

In conclusion it can be said that there is interaction between nucleic acid base and NaOH or piperidine only in protic solvents where NuB acting as proton donor. This donor-acceptor interaction is absent only in the case of AmU only when ethylene glycol is used as solvent. This indicates that solvent must have an important role in this donor-acceptor interaction.

Acknowledgment

One of the authors, Samaresh Mukherjee thanks University College of Dublin for providing him with laboratory facilities in the Department of Chemistry. Thanks are also due to IACS for granting leave and to Mrs. Sarbani Saha for preparing the manuscript.

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